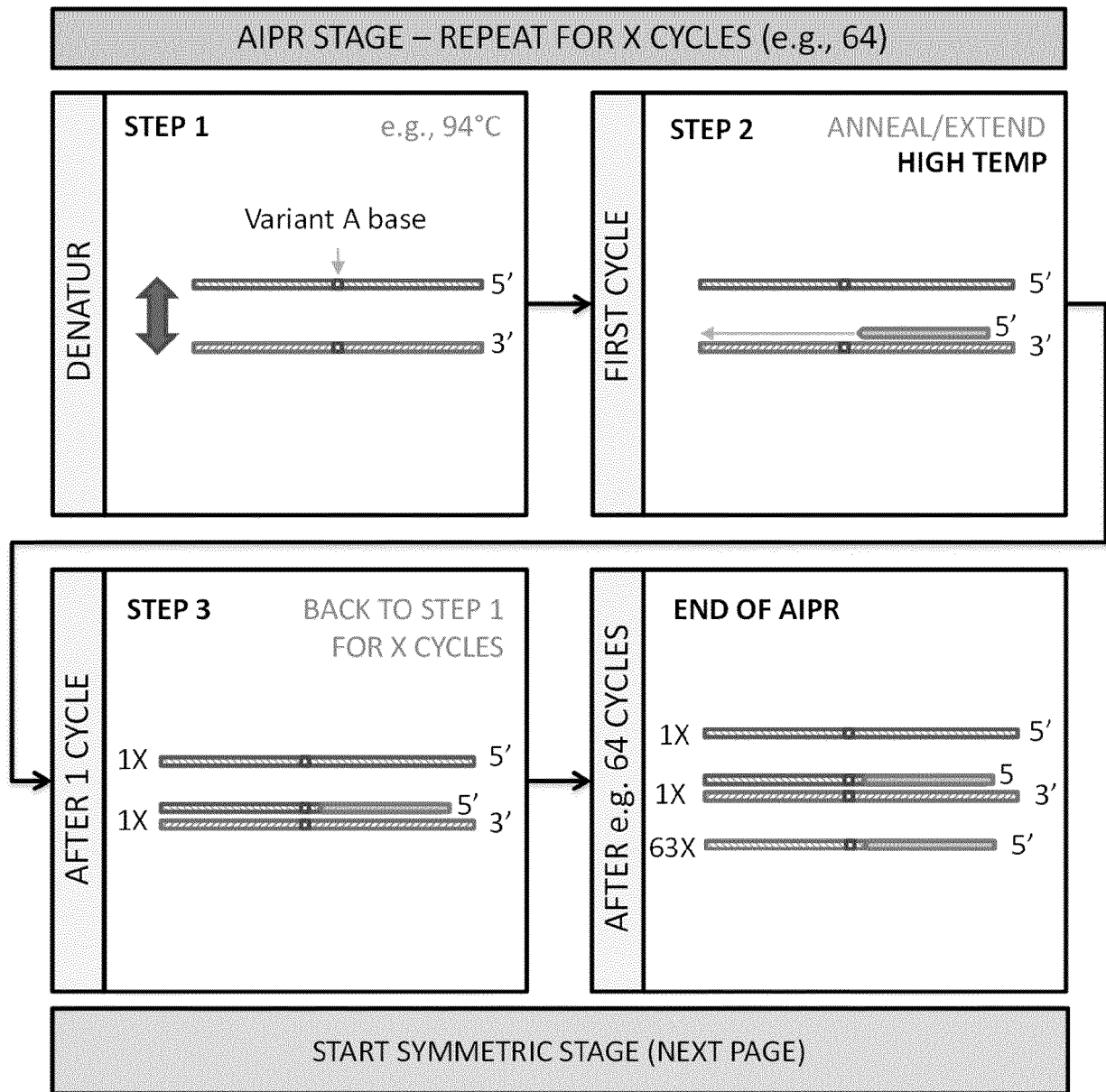


**LEGEND:**

Variant A base	Primer-H	Target Sequence (Plus-Strand)	Target Sequence (Minus-Strand)
■	5'	3' 5'	5' 3'

Fig. 1A

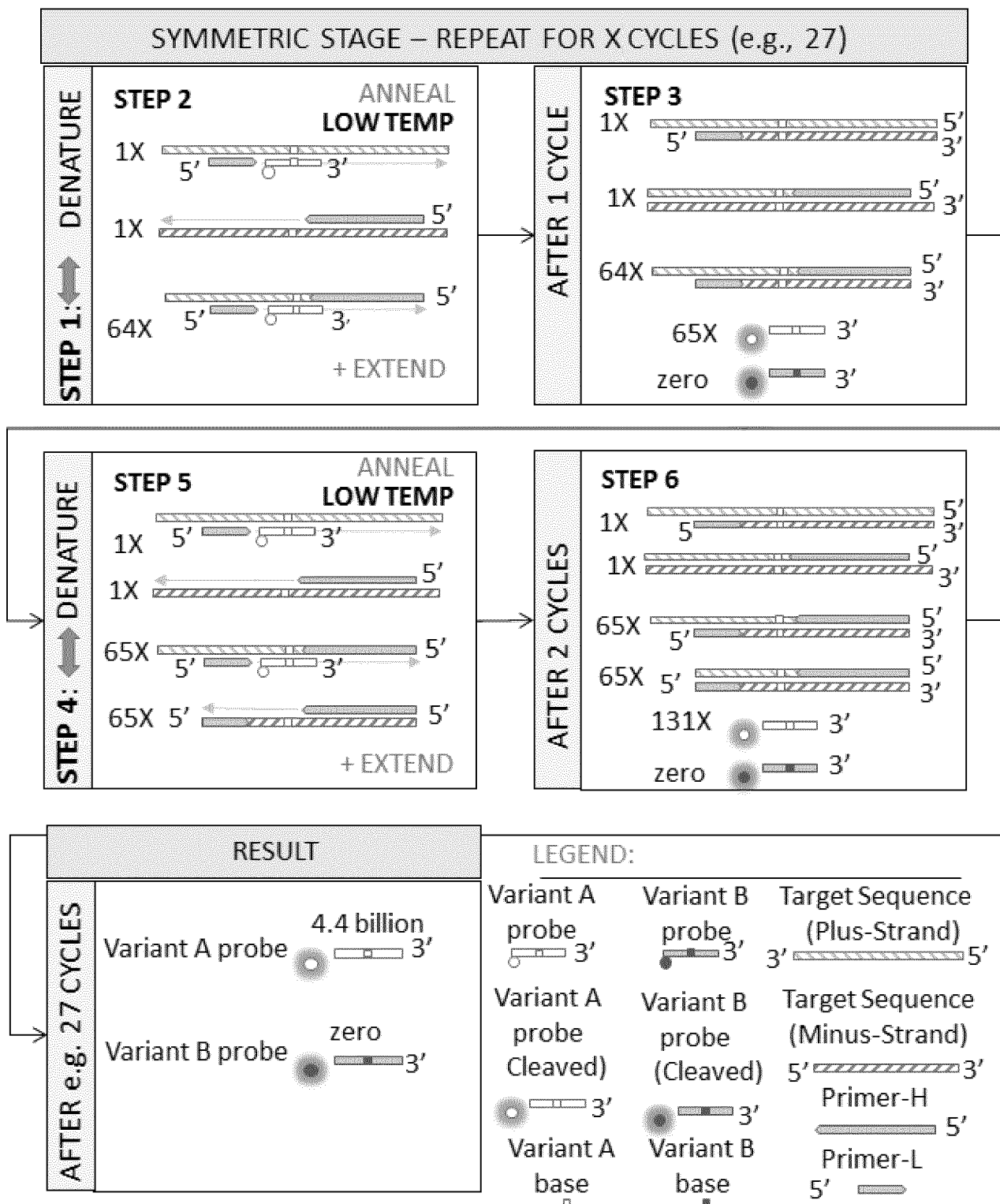


Fig. 1A continued

3/22

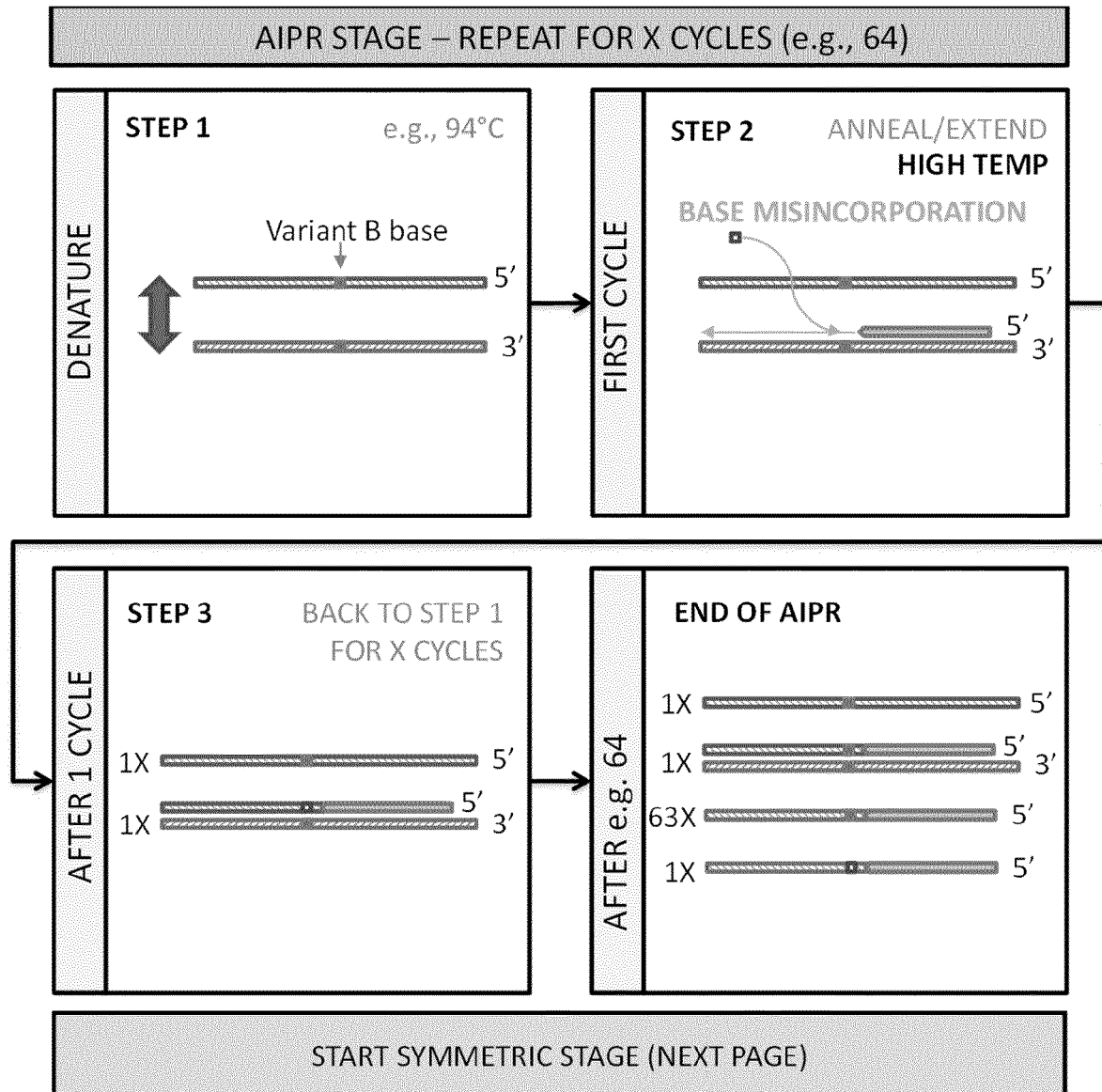
**LEGEND:**Variant A
baseVariant B
basePrimer-H
5'Target Sequence
(Plus-Strand)Target Sequence
(Minus-Strand)

Fig. 1B

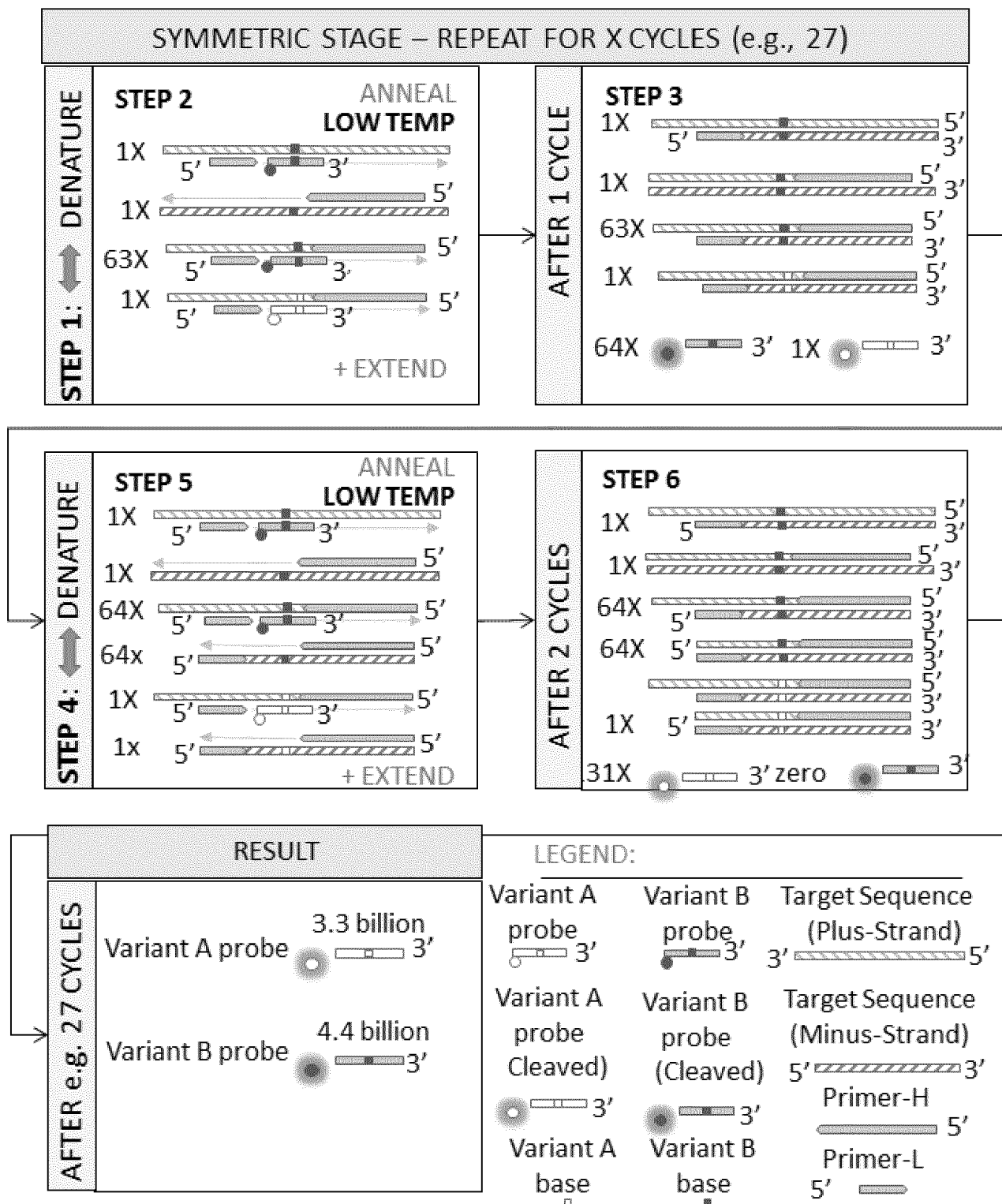


Fig. 1B continued

5/22

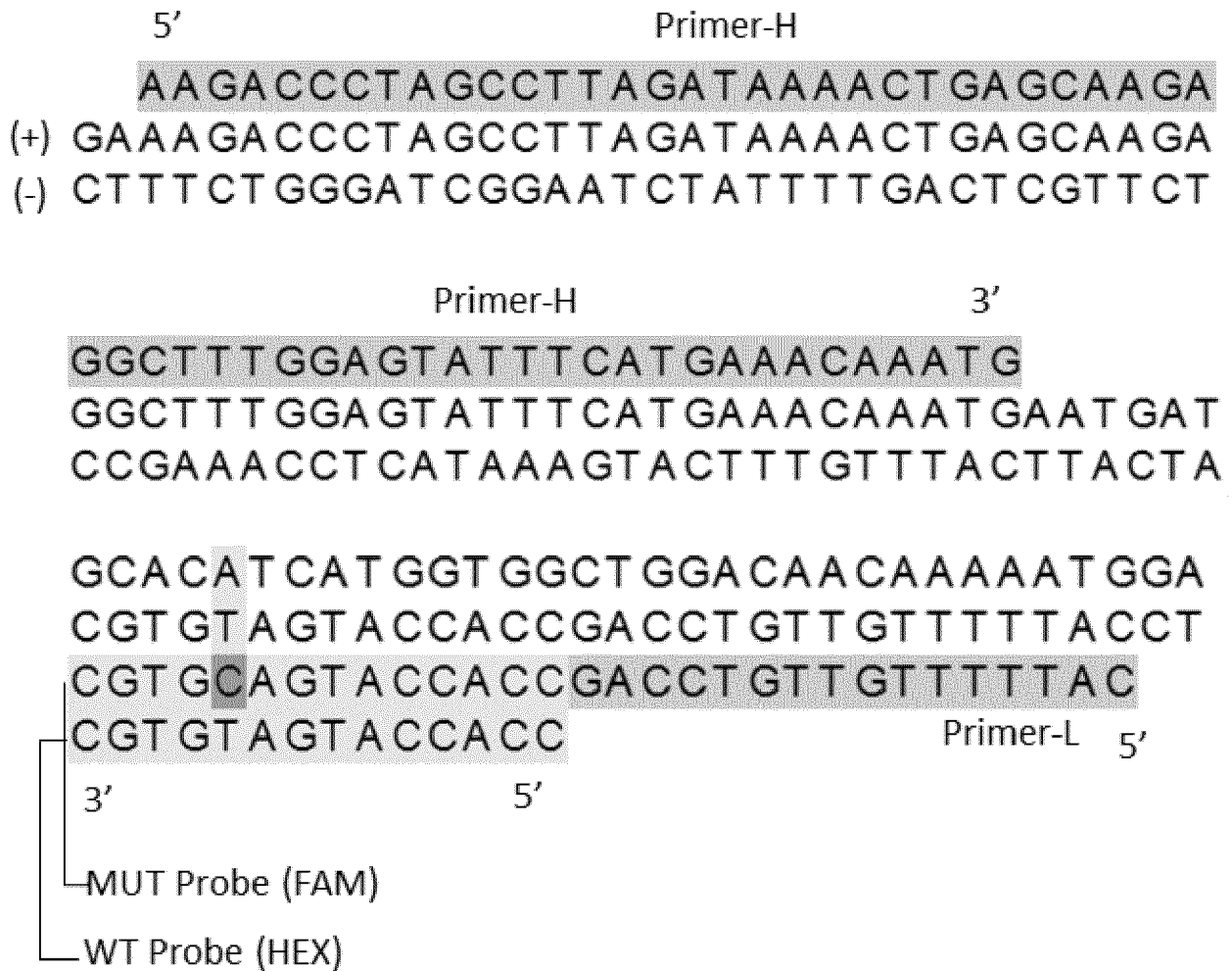
PIK3CA c.3140A>G (H1047R)

Fig. 2

6/22

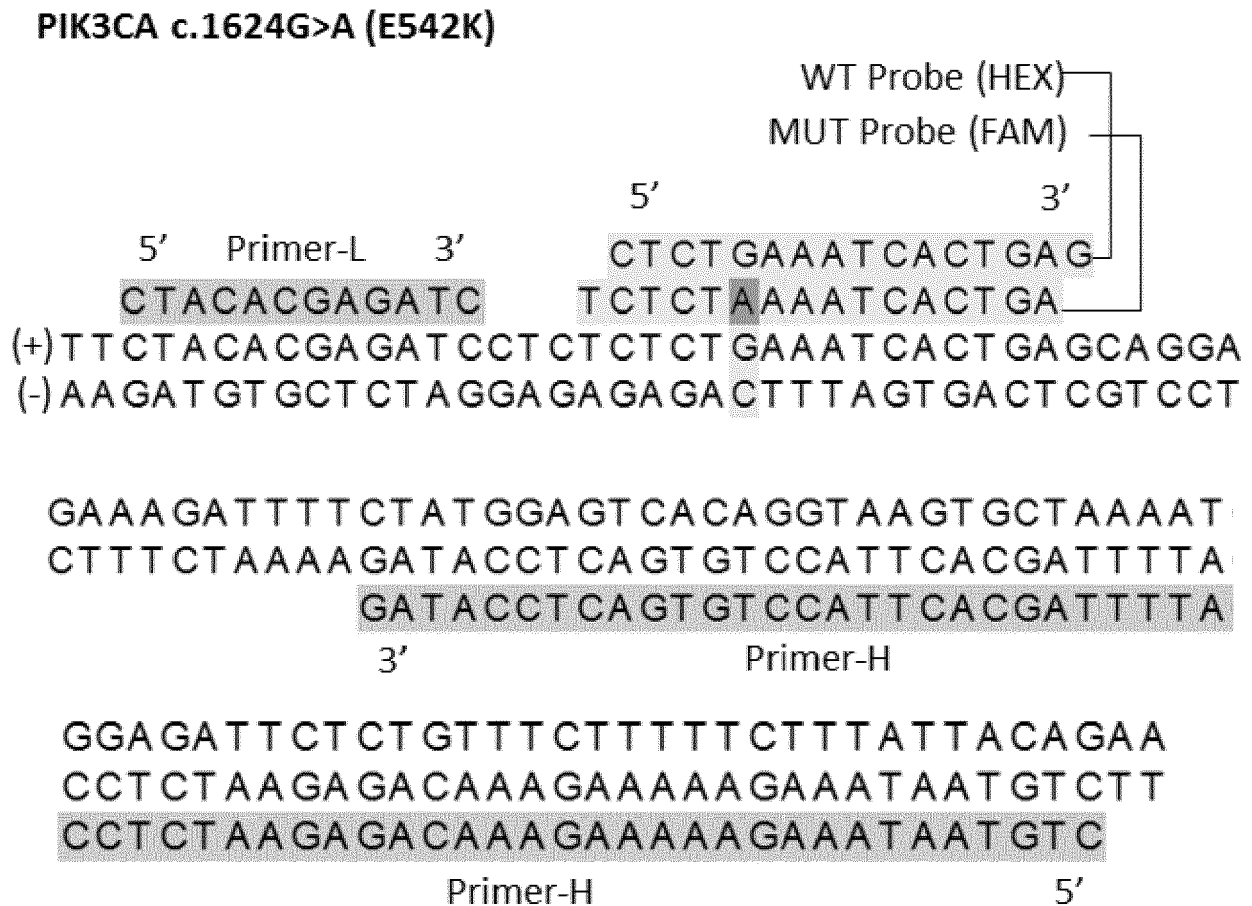


Fig. 2 - Continued

7/22

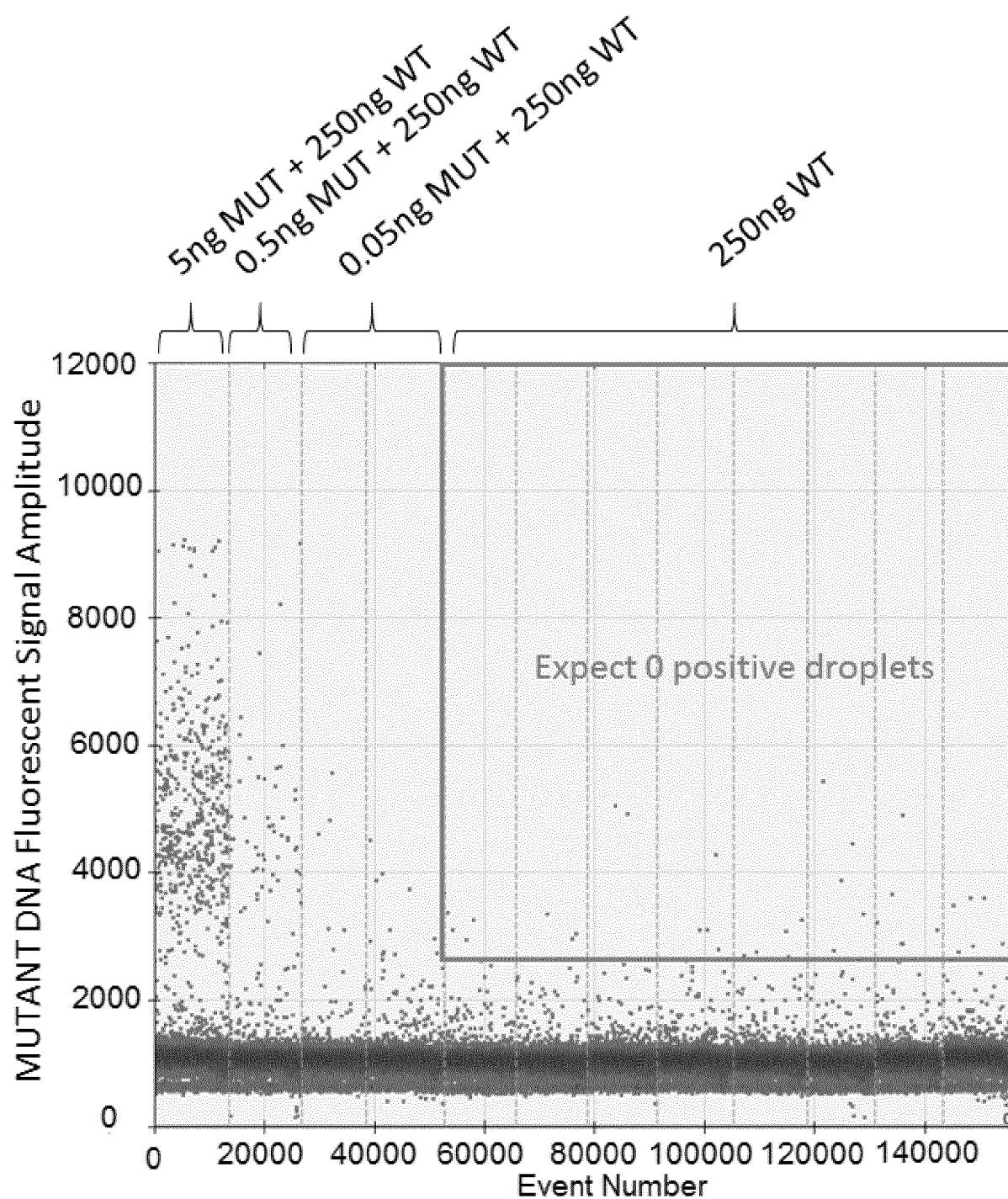


Fig. 3A

8/22

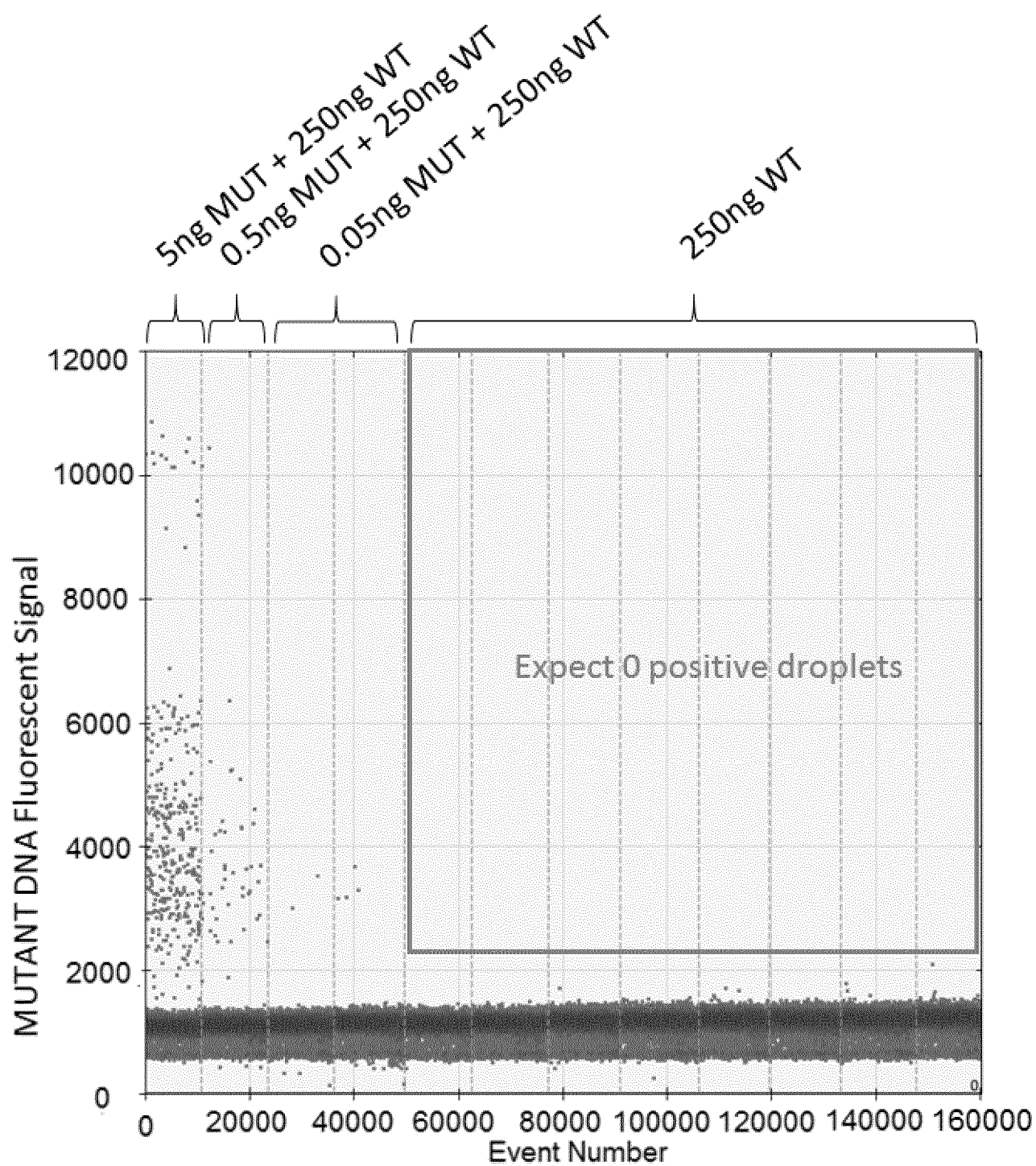


Fig. 3B

9/22

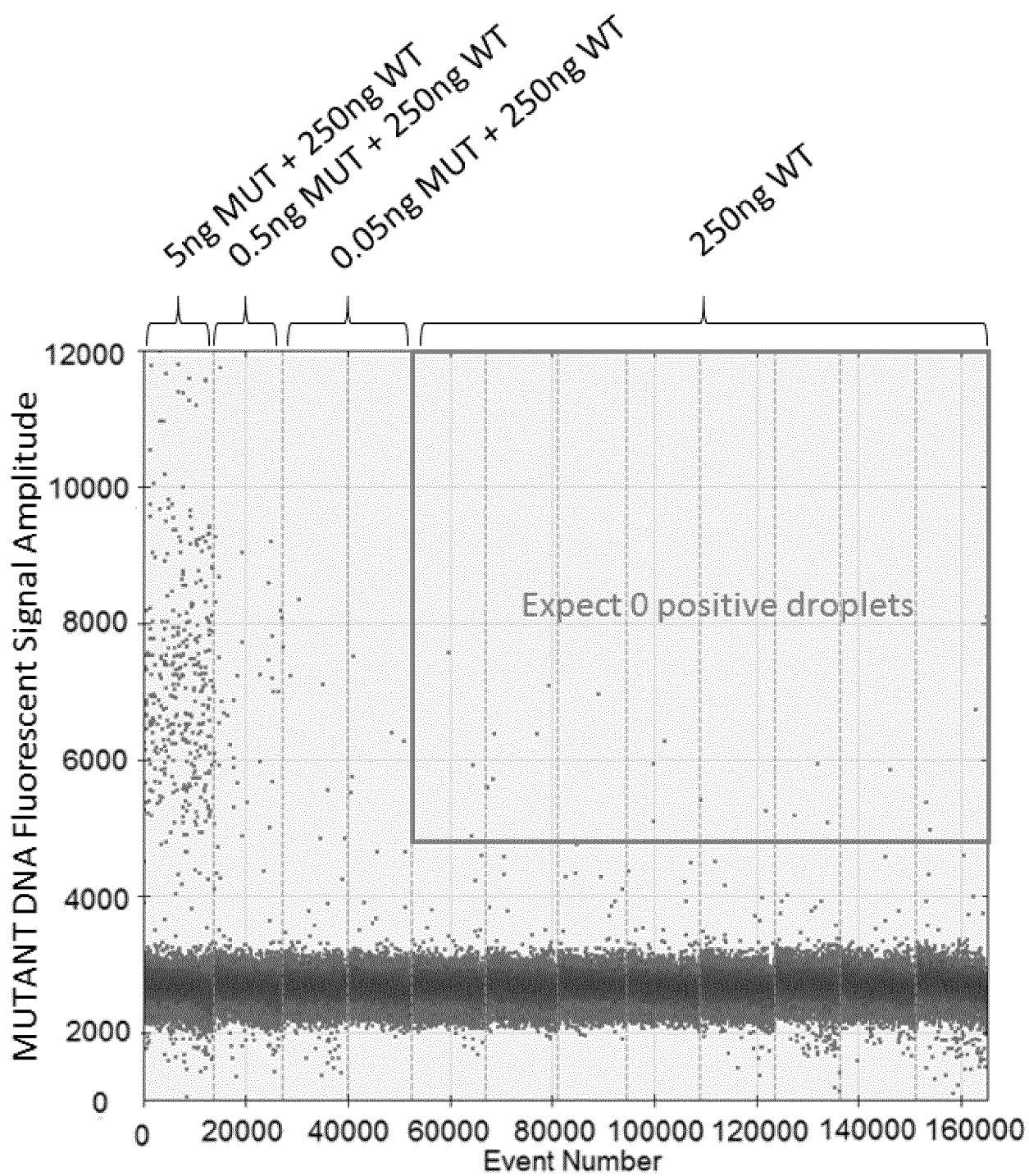


Fig. 3C

10/22

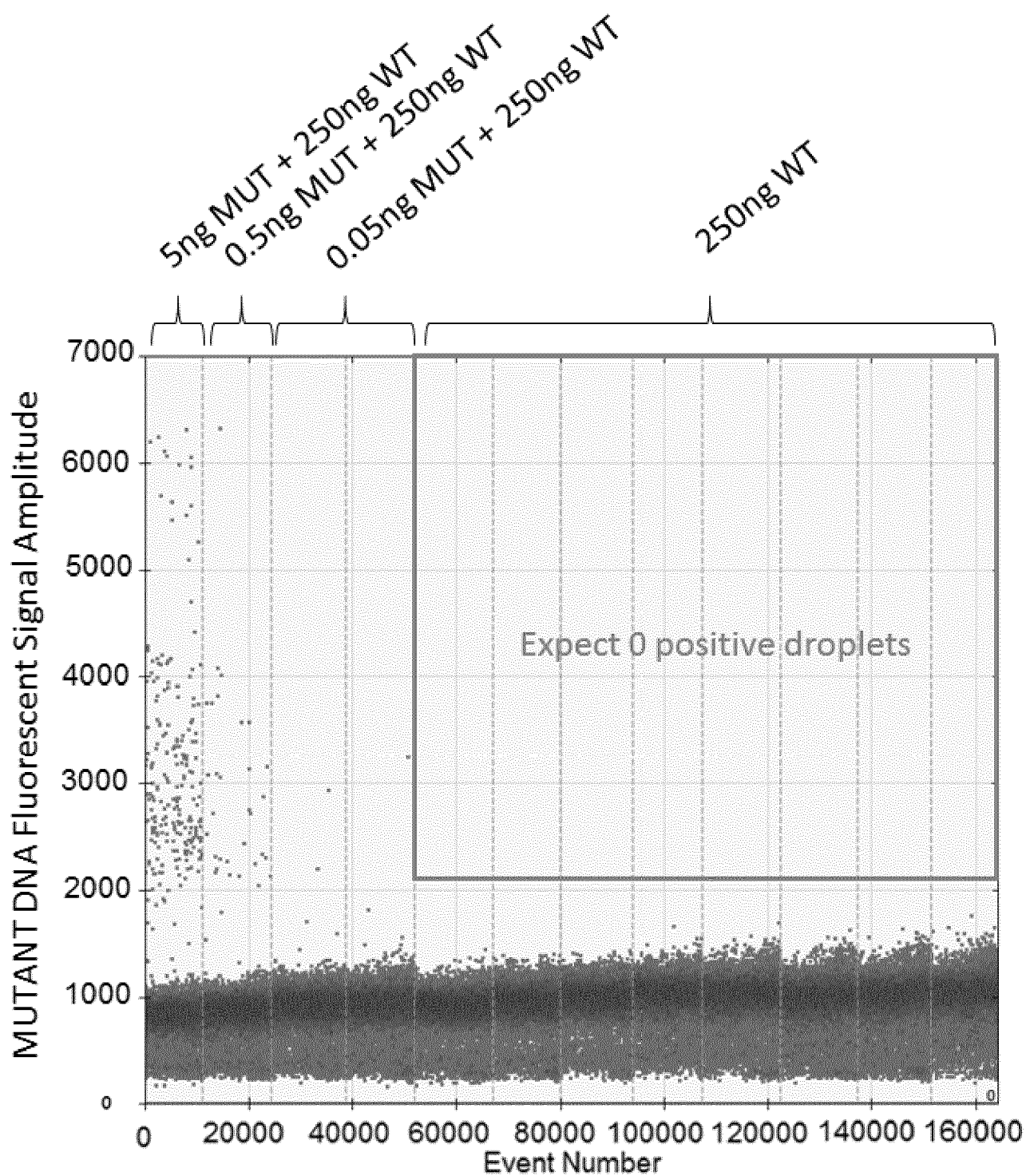


Fig. 3D

11/22

5' CTGAGCAAGAGGCTTT

5' TAGCCTTAGATAAAACTGAGCAAGAGGCTTT

(+ strand CCTAGCCTTAGATAAAACTGAGCAAGAGGCTTT

(-) strand GGATCGGAATCTATTTTGA CTCTCGTTCTCCGAAA

GGAGTATTTTCATGAAACAAATG

Primer-H (beta1)

GGAGTATTTTCATGAAACAAATGAATGATG

Primer-H (beta2)

GGAGTATTTTCATGAAACAAATGAATGATGCACATCATGGTGG
CCTCATAAAGTACTTTGTTTACTTACTACGTGTAGTACCACCMUT Probe (FAM) ACGTGCAGTACCACC
3' 5'WT Probe (HEX) ACGTGTAGTACCACC
3' 5'

CTGGACAACAAAAATGGATTGGATC (+) strand

GACCTGTTGTTTTTACCTAACCTAG (-) strand

GTTGTTTTTACCTAACCT

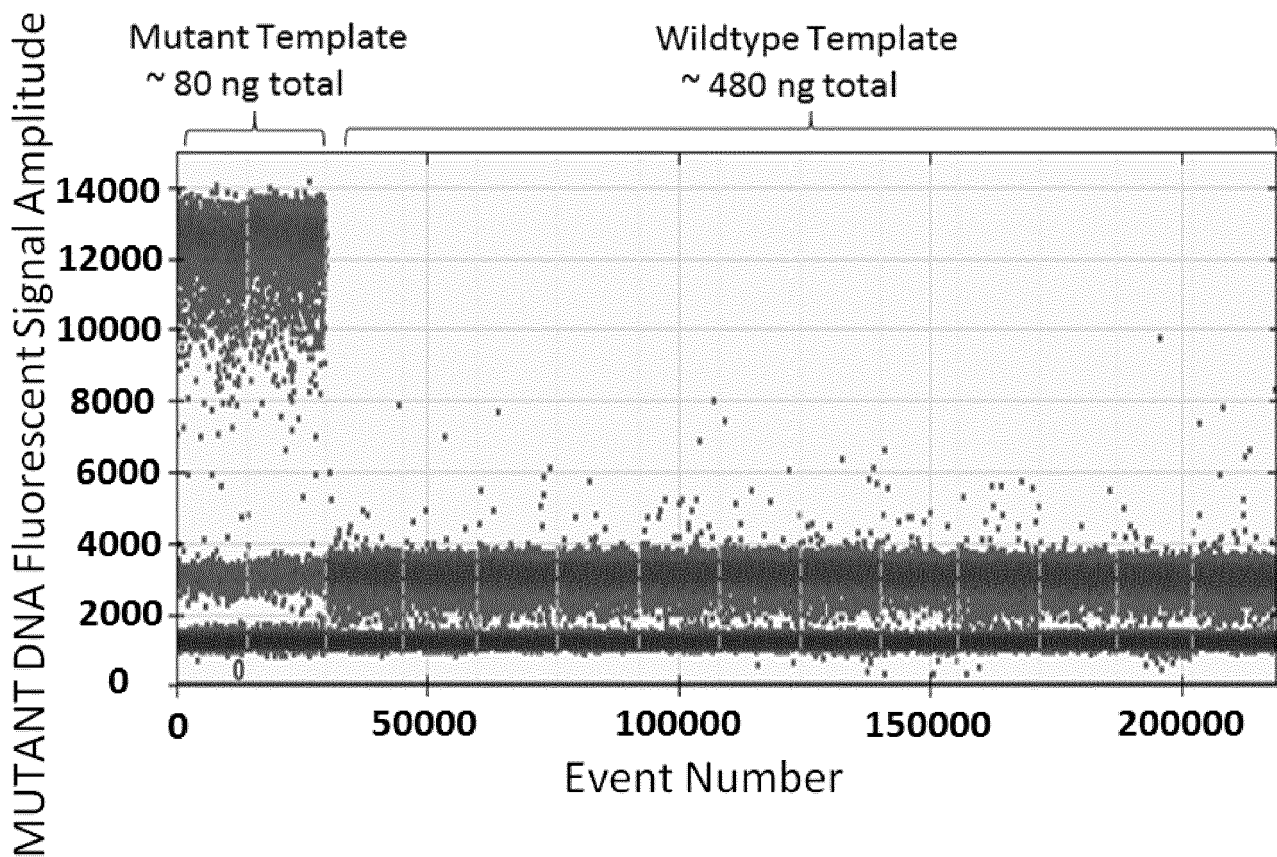
3'

Primer-L

5'

Fig. 4

12/22

**THERMOCYCLER PROGRAM:**

95° C – 10 min

NO AIPR

Repeat 35 cycles:

94° C – 10 sec

52° C – 30 sec

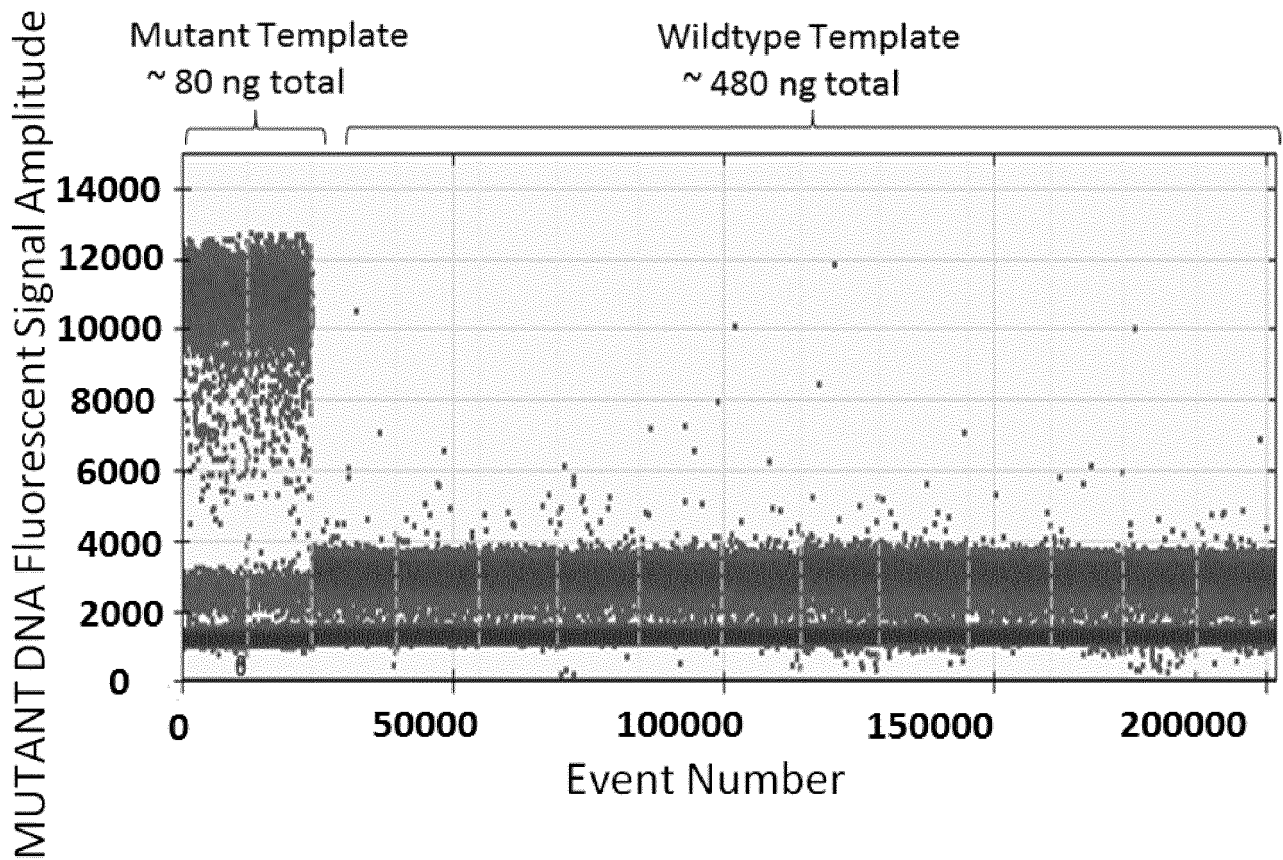
72° C – 30 sec

98° C – 10 min

**SYMMETRIC
EXPONENTIAL
PCR**

Fig. 5A

13/22

**THERMOCYCLER PROGRAM:**

95° C – 10 min

Repeat 64 cycles:

94° C – 10 sec

67° C – 45 sec

AIPR

Repeat 25 cycles:

94° C – 10 sec

52° C – 30 sec

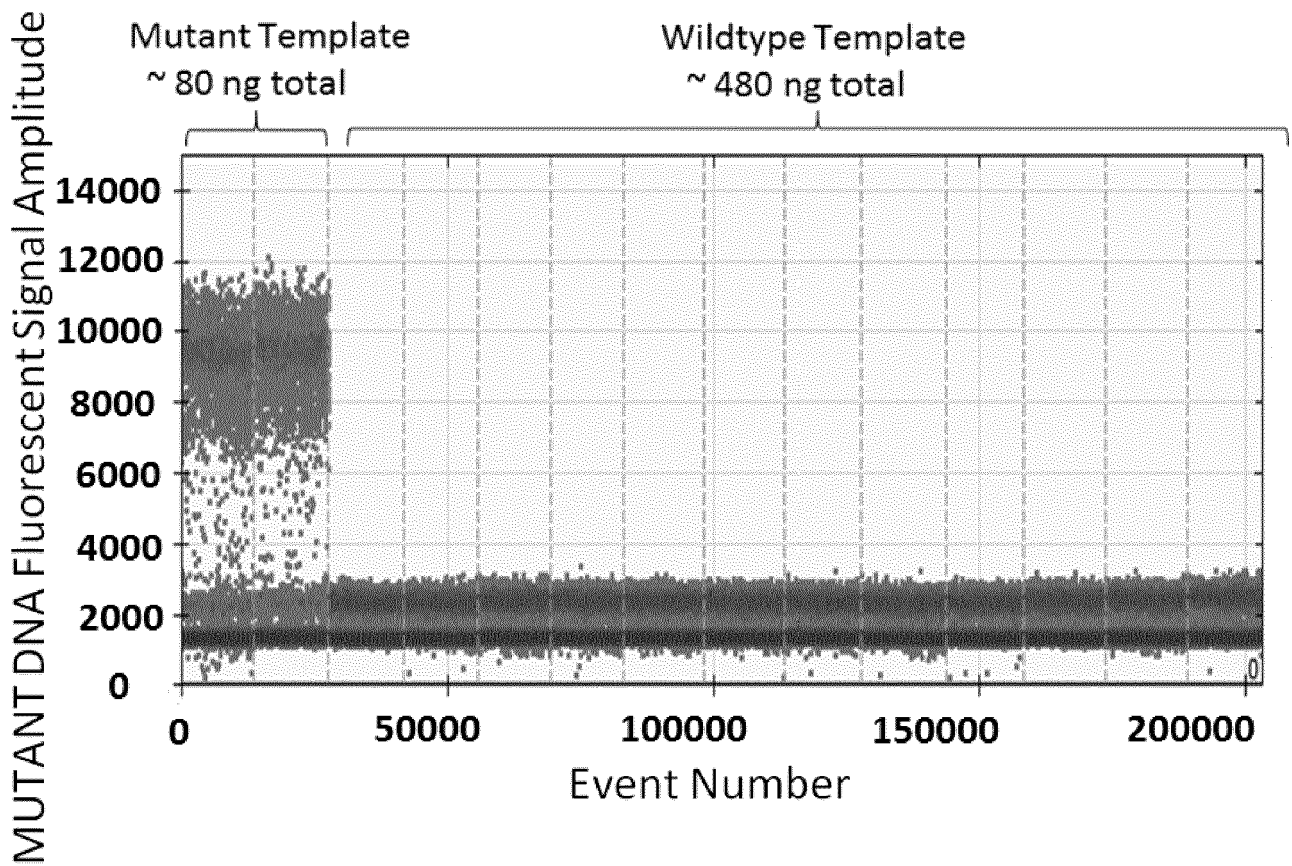
72° C – 30 sec

SYMMETRIC
EXPONENTIAL
PCR

98° C – 10 min

Fig. 5B

14/22

**THERMOCYCLER PROGRAM:**

95° C – 10 min

Repeat 64 cycles:

94° C – 10 sec

74° C – 45 sec

AIPR

Repeat 25 cycles:

94° C – 10 sec

52° C – 30 sec

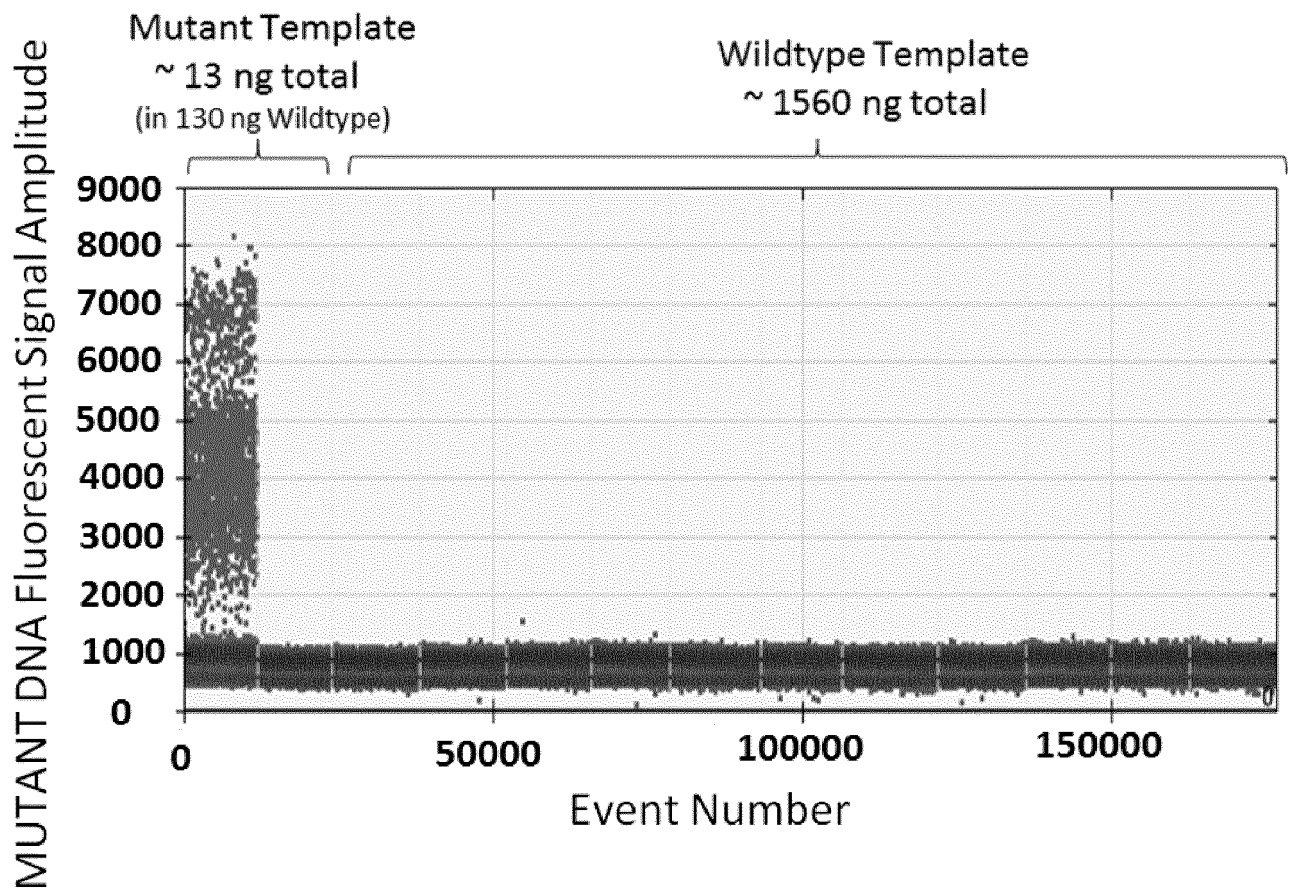
72° C – 30 sec

SYMMETRIC
EXPONENTIAL
PCR

98° C – 10 min

Fig. 5C

15/22

**THERMOCYCLER PROGRAM:**

95°C – 10 min

Repeat **64** cycles:

94°C – 10 sec

75° C – 45 sec

AIPRRepeat **27** cycles:

94°C – 10 sec

48° C – 30 sec

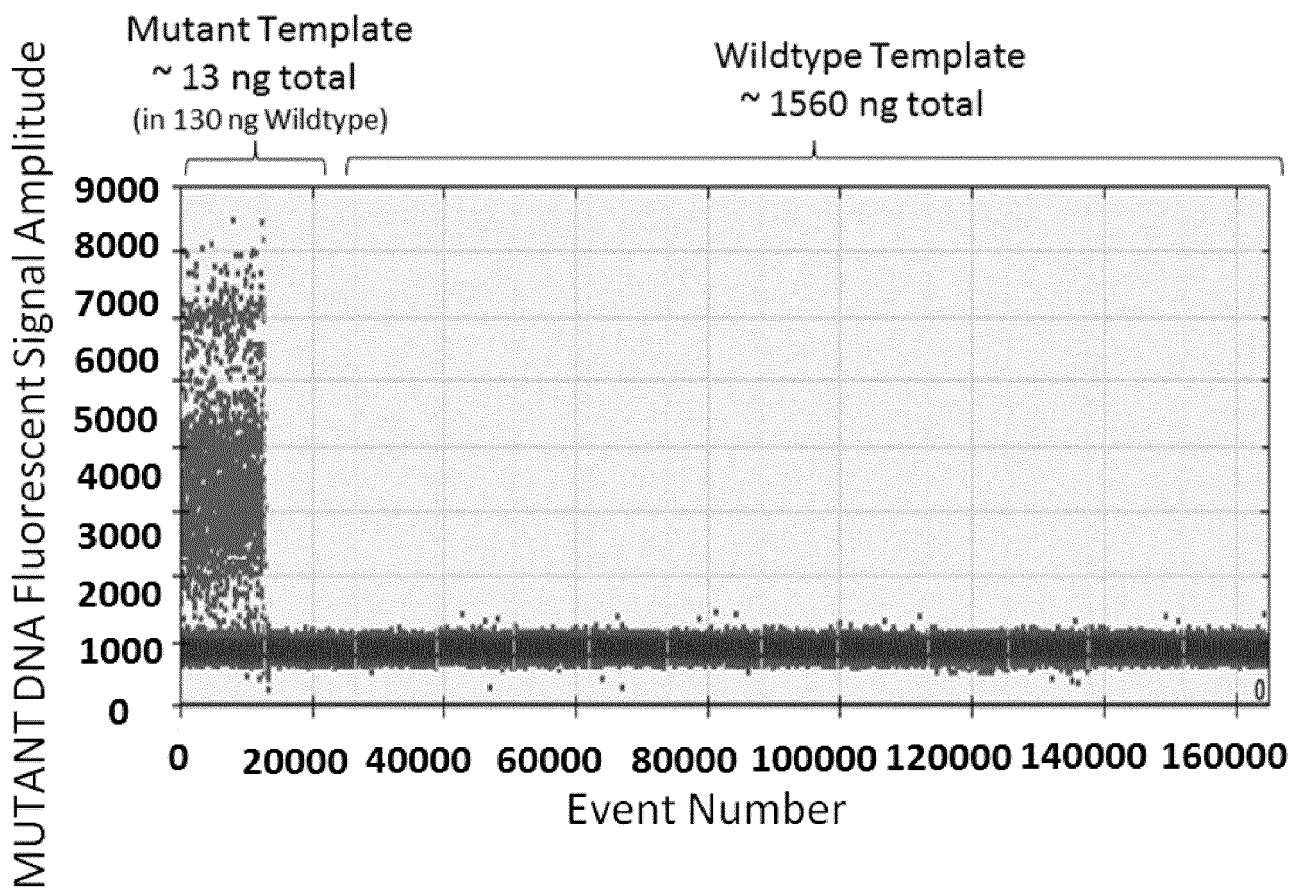
72°C – 30 sec

**SYMMETRIC
EXPONENTIAL
PCR**

98°C – 10 min

Fig. 5D

16/22

**THERMOCYCLER PROGRAM:**

95°C – 10 min

Repeat 64 cycles:

94°C – 10 sec

75°C – 45 sec

AIPR

Repeat 27 cycles:

94°C – 10 sec

46°C – 30 sec

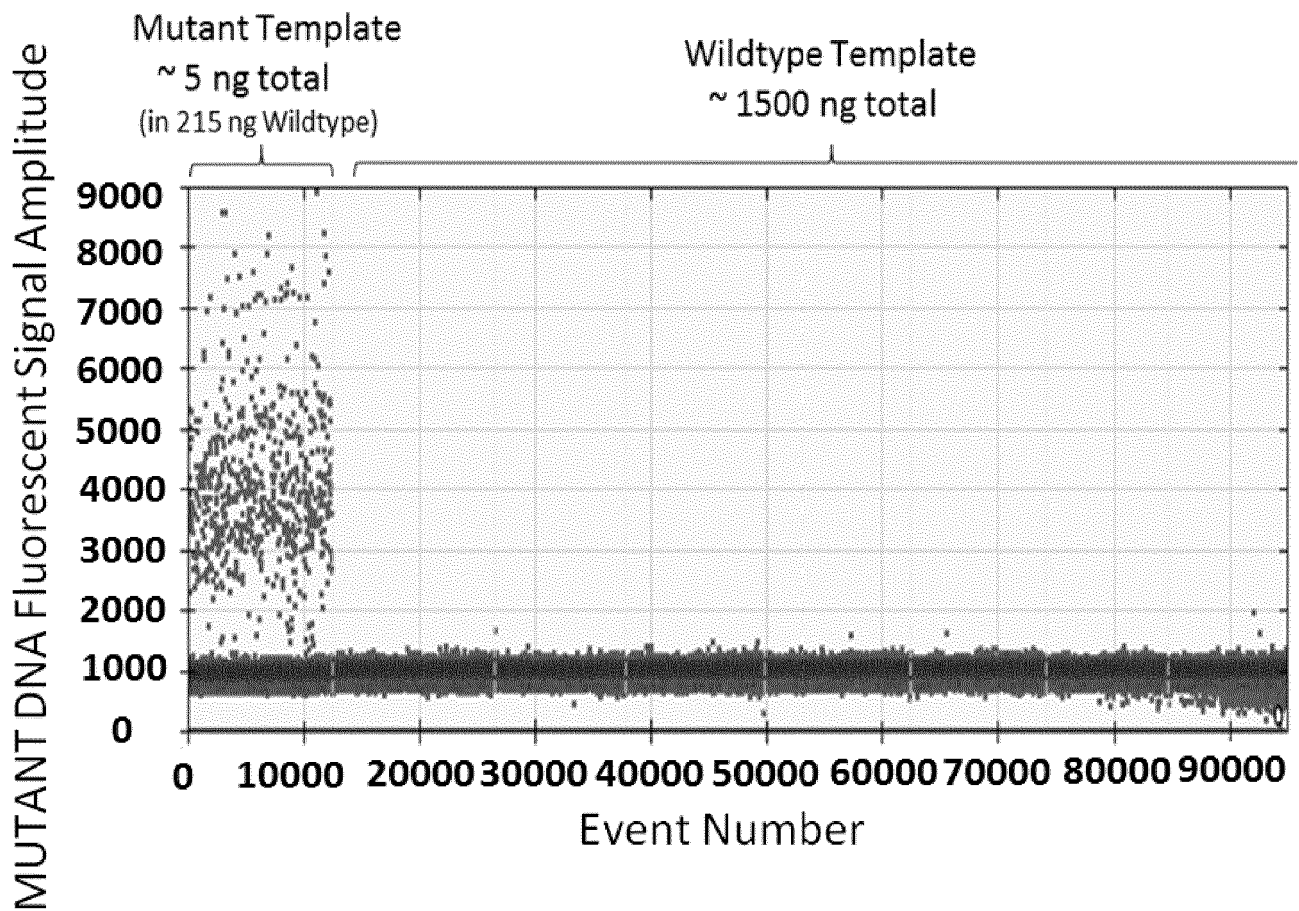
72°C – 30 sec

SYMMETRIC
EXPONENTIAL
PCR

98°C – 10 min

Fig. 5E

17/22

**THERMOCYCLER PROGRAM:**

95°C – 10 min

Repeat 64 cycles:

94°C – 10 sec

73°C – 45 sec

AIPR

Repeat 27 cycles:

94°C – 10 sec

48°C – 30 sec

72°C – 30 sec

98°C – 10 min

SYMMETRIC
EXPONENTIAL
PCR

Fig. 5F

18/22

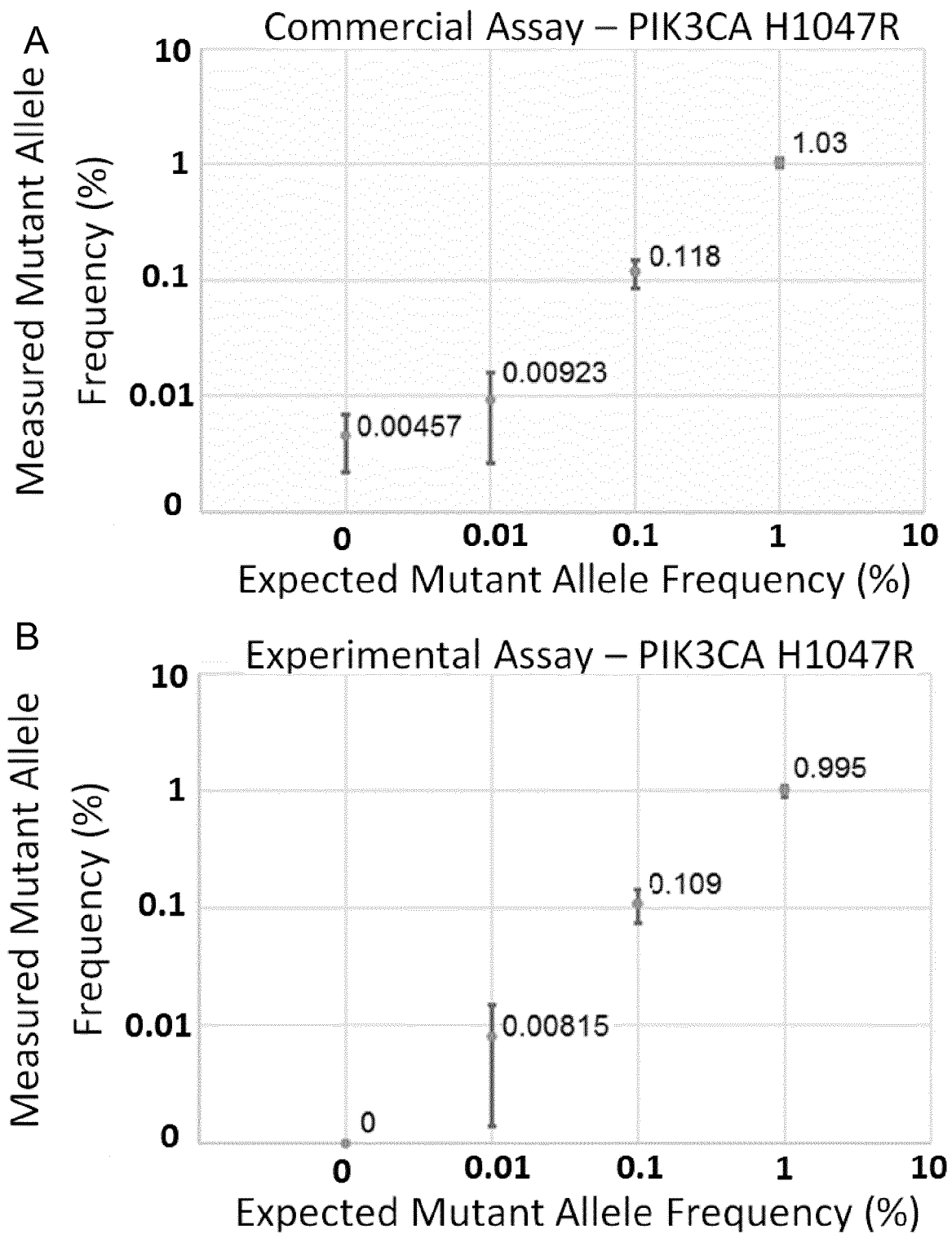


Fig. 6A-B

19/22

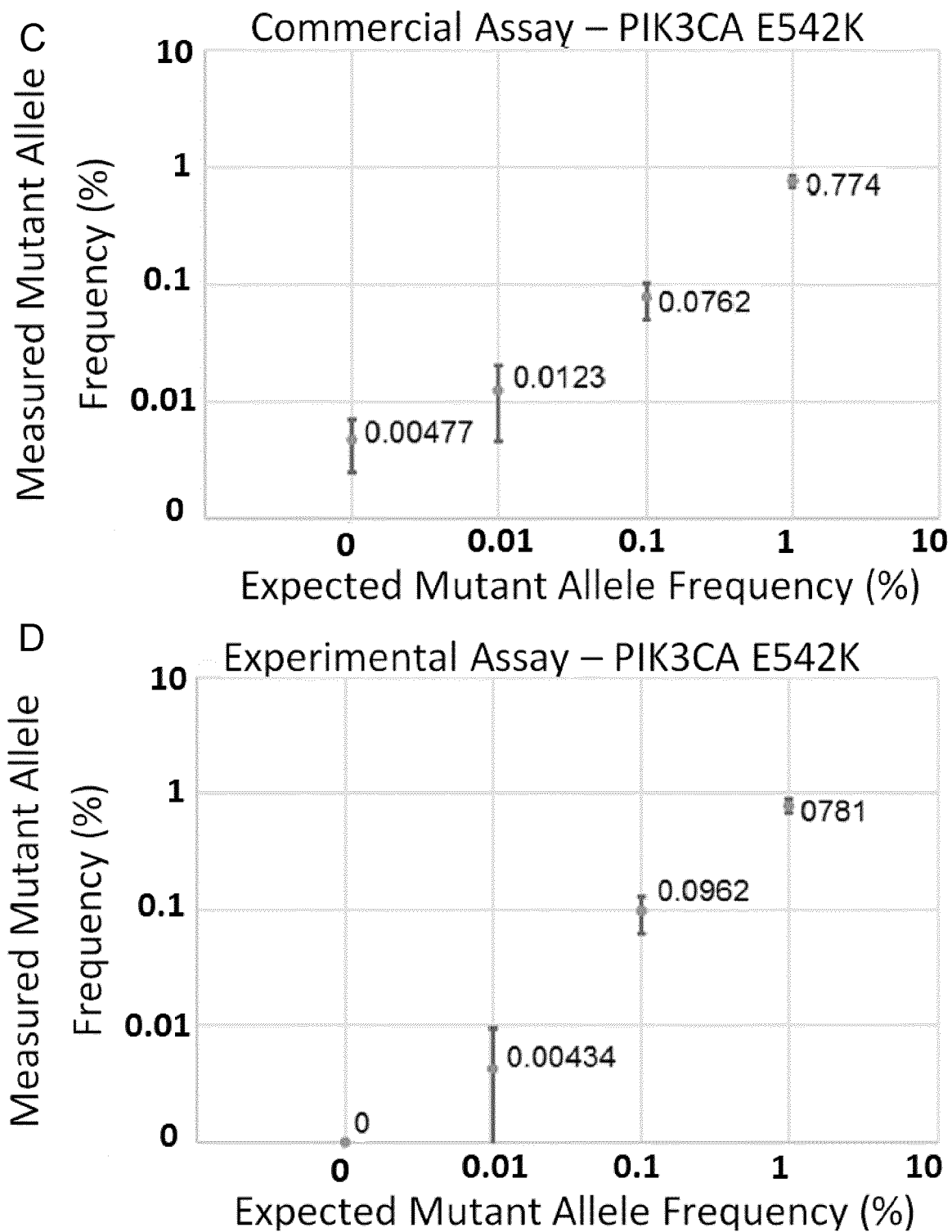


Fig. 6C-D

20/22

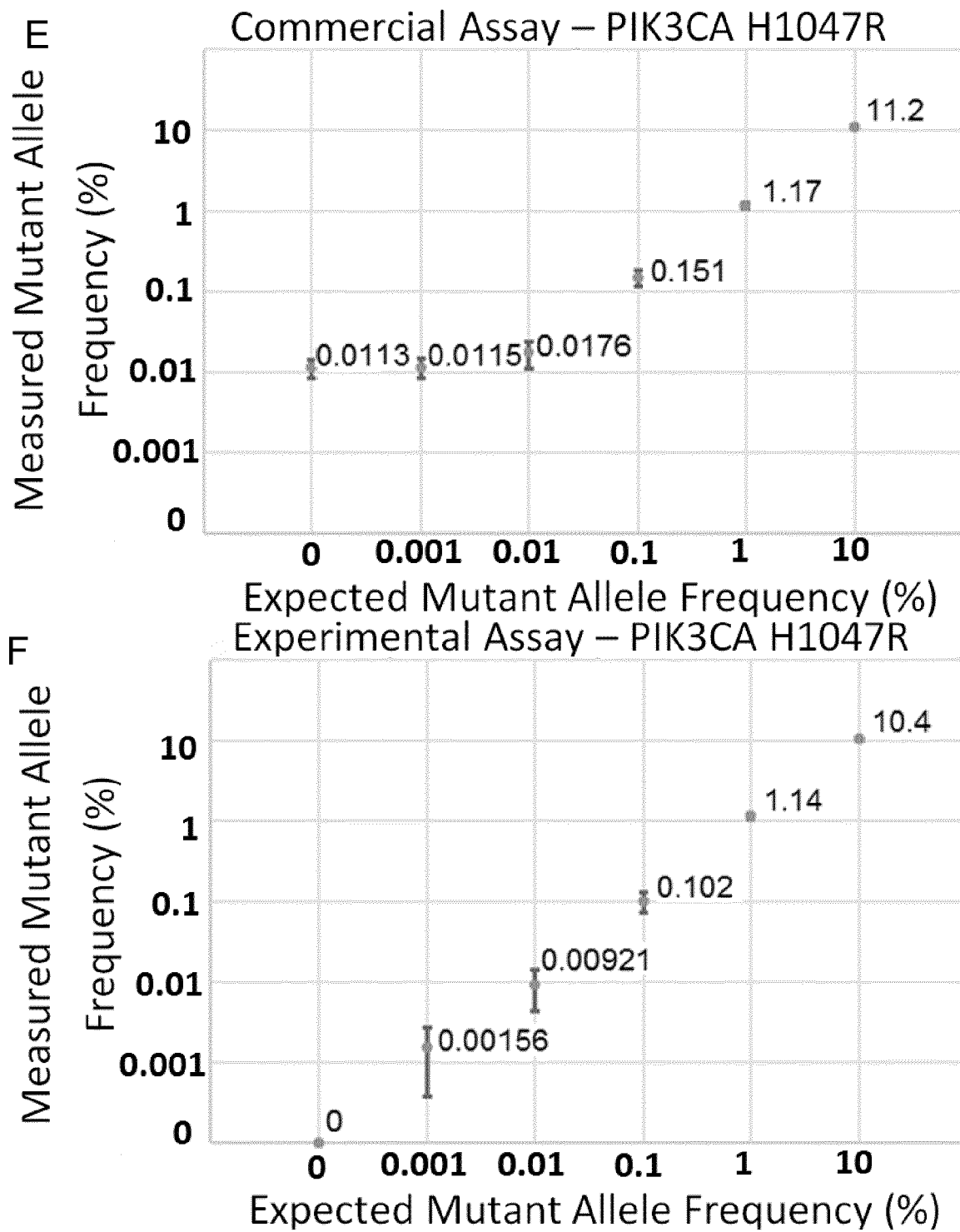


Fig. 6E-F

"mismatch modified
primer-L"

mismatched nucleotides	matched nucleotides
---------------------------	------------------------

5' CCTCAAACCTTGTTGGTAG 3'

(+) strand ATGACTGAATATAAACTTGTGGTAGTTGGAG
(-) strand TACTGACTTATATTTGAACACCATCAACCTC

5' CTGGTGGCGTAGG 3' WT Probe (HEX)

5' CTGGTGACGTAGGCAA 3' MUT Probe (FAM)

CTGGTGCGTAGGCAAGAGTGCCTTGACGATACAGCTAA
GACCACCGCATCCGTTCTCACGGAAGTCTGCTATGTCGATT
CTCGCGGAAGTCTGCTATGTCGATT

3' Primer-H

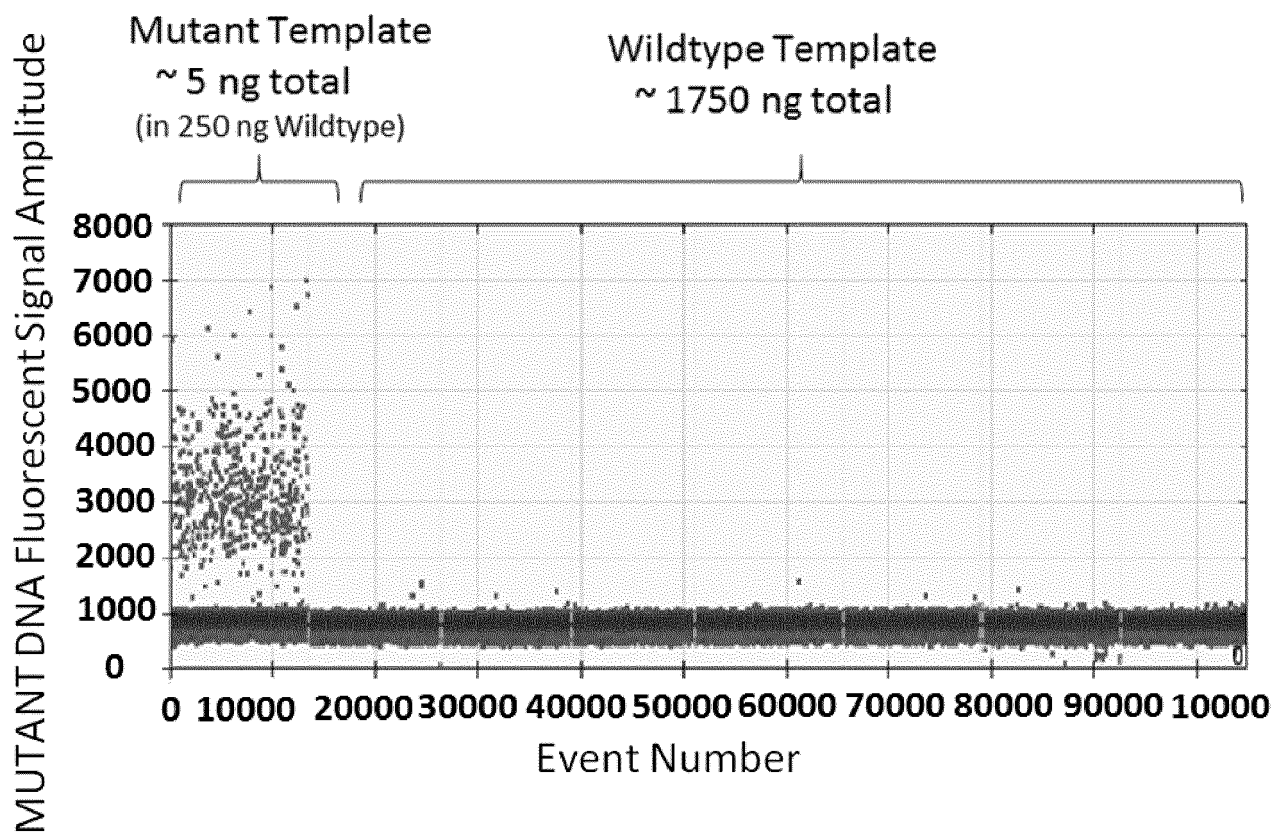
TTCAGAATCATTTTGTGGACGAATATGATCCAACAAT
AAGTCTTAGTAAACACCTGCTTATACTAGGTTGTTA|

AAGTCTTAGTAAACACCTGCTTATACTAGGTTGTTA

Primer-H 5'

Fig. 7A

22/22

**THERMOCYCLER PROGRAM:**

95°C – 10 min

Repeat 64 cycles:

94°C – 10 sec

73°C – 45 sec

AIPR

Repeat 5 cycles:

94°C – 10 sec

30°C – 30 sec

72°C – 30 sec

LOW

TEMPERATURE

PCR

Repeat 22 cycles:

94°C – 10 sec

53°C – 30 sec

72°C – 30 sec

98°C – 10 min

SYMMETRIC

EXPONENTIAL

PCR

Fig. 7B